

In vitro antimicrobial activity of mouth washes and herbal products against dental biofilm-forming bacteria

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Abstract

Aim: To evaluate *in vitro*, the antimicrobial effect of *Cymbopogon citrates* (lemon grass), *Plectranthusamboinicus* (Mexican mint) and *Conyzabonariensis* (hairy fleabane) tinctures as well as pure and diluted commercial mouth washes (Malvatricin®, Periogard® and Listerine®) on wild isolates of *Streptococcusmutans* and reference strains of *S. mutans*, *Streptococcus salivarius*, *Streptococcus oralis* and *Lactobacillus casei* by determination of minimum inhibitory dilution (MID). **Materials and Methods:** 0.12% chlorhexidine and 70% corn alcohol were used as positive and negative controls, respectively. Saliva samples were collected from 3 volunteers and seeded in MSB broth to obtain *Streptococcus* isolates after 72-hour incubation. Using the agar diffusion method, susceptibility tests were performed with overnight incubation in microaerophilia at 37°C. All tests were performed in duplicate. **Results:** The bacterial species were resistant to the tinctures and Listerine®, but were susceptible to 0.12% chlorhexidine, Malvatricin® and Periogard®, with MIDs ranging from 12.5% to 1.56%. **Conclusions:** *Plectranthusamboinicus*, *Conyzabonariensis* and *Cymbopogoncitratatus* tinctures and Listerine® did not show inhibitory action against the tested biofilm-forming bacteria.

Keywords: Dental plaque, mouthwashes, phytotherapy, plant extracts

Introduction

Herbal products have recently undergone more thorough investigation for their potential in preventing oral diseases, particularly plaque-related diseases, such as dental caries.^[1-3] It is well known that *Streptococcus mutans* and other cariogenic bacteria are the major etiological agents in dental caries.^[4,5] Insoluble glucans synthesized by *S. mutans* increase the pathogenicity of oral biofilm by promoting the adherence and accumulation of cariogenic bacteria on tooth surface.^[6]

Mechanical removal of dental biofilm is a key factor in the prevention of oral diseases and may be either associated or not to the use of agents that act specifically against cariogenic microorganisms.^[7] Among the currently available antiseptics,

0.12% chlorhexidine is the most commonly indicated substance because this concentration provides antimicrobial efficacy with less severe adverse effects.^[8,9] Twelve percent chlorhexidine is the principal ingredient of Periogard®.

Malvatricin® mouth wash is an association of antiseptic and decongestant substances, like tyrothricin and mallow hidrolato, which are responsible for the bacteriostatic action of this product, mainly against gram-positive bacteria.^[10] Listerine® is a product based on essential oils, like thymol and eucalyptol, which act nonspecifically against bacteria and are widely used as disinfectants and antiseptics, but may cause burning sensation and oral tissue staining.^[11]

The use of natural antimicrobials may contribute to control the disordered growth of oral microbiota, thus overcoming problems caused by species resistant to conventional antimicrobials.^[12,13] Natural substances have demonstrated antibacterial action mainly because most plants used in alternative medicine are composed of flavonoids, which act on bacterial cells disrupting the cytoplasmatic membrane and inhibiting the enzymatic activity.^[13]

The specie *Cymbopogoncitratatus* (D.C.) Stapf., belongs to the Poaceae family and is popularly know as lemon grass, being widely used due to its antibacterial, antigenotoxic and antiinflammatory actions.^[14] *Plectranthusamboinicus* (Mexican mint) is a plant from the *Coleus* family that is used because of its anticough, antimicrobial and antioxidant effects, the latter being attributed to the flavonoids present in its chemical composition. *Conyzabonariensis* (hairy fleabane) is native from South America and is used in the treatment of rheumatism, gout and nephritis.

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The purpose of this study was to evaluate *in vitro*, the antimicrobial effect of herbal products and commercial mouth washes against wild isolates and reference strains of dental biofilm-forming bacteria by determination of minimum inhibitory dilution (MID).

Material and Methods

This study was conducted in compliance with the ethical guidelines issued by the Resolution 196/96 of the Brazilian National Health Council/Ministry of Health on research involving human subjects. The research project was independently reviewed and approved by the Ethics in Research Committee of the Federal University of Paraíba, Brazil (Protocol number 562/06).

Wild species of *S. mutans* isolated from saliva samples and reference strains of *S. mutans* (ATCC 25175), *Streptococcus salivarius* (ATCC 7073), *Streptococcus oralis* (ATCC 10557) and *Lactobacillus casei* (ATCC 9595) were selected for being among the most prevalent oral microorganisms.

Three volunteers participated in the study. Non-stimulated whole saliva was collected in the morning between 8 and 9 a.m.. No oral hygiene was allowed before collection. Approximately 3 mL of saliva were collected from each participant in sterile plastic tubes and seeded in *Mitisalivarius* bacitracin (MSB) agar. All volunteers were fully informed on the study purposes and signed an informed consent form.

The antimicrobial activity of Periogard® (Colgate-Palmolive Co., New York, NY, USA), Listerine® (Johnson & Johnson, Somerville, NJ, USA) and Malvatricin® (Laboratório Daudt Oliveira Ltda., Rio de Janeiro, RJ, Brazil) mouth washes, and *Cymbopogon citratus*, *Plectranthus amboinicus* and *Conyzabonariensis* tinctures was evaluated. The commercial mouthrinses and herbal products were evaluated in their pure form as well as in six dilutions

ranging from 1:2 to 1:64. The positive and negative controls were 0.12% chlorhexidine and 70% corn alcohol (used in the preparation of tinctures), respectively.

The bacterial strains were reactivated in Brain Heart Infusion (BHI) broth and incubated overnight in microaerophilia at 37°C. Next, the MIDs were determined by the agar diffusion method. The inocula were seeded in Petri dishes containing Muller Hinton agar with 5% blood using swabs and approximately 6-mm-diameter wells were further perforated and filled with 50 µL of the dilutions. The dishes were incubated in microaerophilia at 37°C for 48 hours.^[15]

MID was considered as being the smallest range of product dilution capable of preventing bacterial growth with formation of inhibition zones measured in millimeters with a digital caliper. All tests were performed in duplicate.

Results

0.12% chlorhexidine presented MIDs between 12.5% and 1.56%, while Malvatricin® presented MIDs ranging from 12.5% to 6.25%. Listerine® and the herbal products derived from *Cymbopogon citratus*, *Plectranthus amboinicus* and *Conyzabonariensis* did not show antibacterial activity against the tested strains [Table 1].

Discussion

The search for substances with antimicrobial activity is a continuous challenge and medicinal plants have been considered an interesting option because of their use in popular medicine to treat several infectious diseases.^[11] The use of plant compounds for pharmaceutical purposes has gradually increased in Brazil.

Several studies have investigated the antimicrobial activity of natural products against oral microorganisms.^[1-3,5]

Table 1: Minimum inhibitory dilutions (MIDs) and the respective zones of bacterial growth inhibition

	<i>S. salivarius</i>	<i>S. oralis</i>	<i>L. casei</i>	<i>S. mutans</i>	<i>S. mutans</i> Volunteer 1	<i>S. mutans</i> Volunteer 2	<i>S. mutans</i> Volunteer 3
Periogard®	3.125% 8 mm	1.56% 8 mm	6.25% 12 mm	6.25% 9.5 mm	*	*	*
Malvatricin®	*	*	6.25% 8 mm	12.5% 8 mm	*	*	*
Listerine®	-	-	-	-	-	-	-
<i>Cymbopogon citratus</i>	-	-	-	-	-	-	-
<i>Plectranthus amboinicus</i>	-	-	-	-	-	-	-
<i>Conyzabonariensis</i>	-	-	-	-	-	-	-
70% Corn Alcohol	-	-	-	-	-	-	-
0.12% chlorhexidine	3.125% 8 mm	12.5% 8 mm	3.125% 8 mm	3.125% 8 mm	*	*	1.56% 12 mm

(*) Overlapped zones of bacterial growth inhibition; (-) No formation of zones of bacterial growth inhibition

However, to the best of our knowledge there are no studies referring to the antimicrobial action of *Cymbopogon citratus*, *Plectranthusamboinicus* and *Conyzabonariensis* against *S.mutans*, *S. salivarius*, *S.oralis* and *L. casei*.

Chlorhexidine is considered a safe and effective antiseptic for reduction of plaque,^[16] gingivitis and *mutans* streptococci levels.^[7] The antimicrobial activity of 0.12% chlorhexidine has been extensively investigated and the findings of the present study are consistent with the literature,^[7,9,17] as Periogard® inhibited the bacterial growth with MIDs varying from 6.25% to 1.56%.

Listerine® mouthrinse is a combination of essential oils (eucalyptol, menthol, thymol and methyl salicylate), which has been proved efficacious for the reduction of dental plaque and gingivitis.^[16] However, in this study Listerine® did not exert inhibitory effect against any of the tested strains, differently from the findings of previous investigation.^[10,18]

Cymbopogon citratus, *Plectranthusamboinicus* and *Conyzabonariensis* tinctures did not show antimicrobial action against the dental biofilm microorganisms. However, it has been demonstrated that even not having an isolated action against specific bacterial species, phytotherapeutic products may act as important adjuvant agents potentializing the effect of other medications.^[12,13,19] Regarding the negative control, 70% corn alcohol did not inhibit the growth of any of the tested bacterial strains, as reported elsewhere.^[20]

Phytotherapy has been widely studied in the last years and may be an important treatment alternative for presenting, among other advantages, low cost and minimal side effects when properly used.^[12,13] Plants with therapeutic properties used in traditional healthcare constitute an important source of new biologically active compounds.^[21] However, changes in the period, region and season at which the plants are collected for preparation of extracts may determine differences in the outcomes.^[22]

In view of the limitations of *in vitro* studies, it is worth mentioning that these results may not correspond to the actual behaviors of tinctures *in vivo* because they are not exposed to the same conditions found in the oral cavity. Nevertheless, laboratorial studies are needed to support the performance of further clinical investigations.

Conclusions

Cymbopogon citratus, *Plectranthusamboinicus* and *Conyzabonariensis* tinctures and Listerine® mouth wash did not show inhibitory action against the tested biofilm-forming bacteria.

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References

1. Yamaguti-Sasaki E, Ito LA, Canteli VC, Ushirobira TM, Ueda-Nakamura T, Dias Filho BP, *et al.* Antioxidant capacity and *in vitro* prevention of dental plaque formation by extracts and condensed tannins of *Paulliniacupana*. *Molecules* 2007;12:1950-63.
2. Smullen J, Koutsou GA, Foster HA, Zumbé A, Storey DM. The antibacterial activity of plant extracts containing polyphenols against *Streptococcus mutans*. *Caries Res* 2007;41:342-9.
3. Muanza DN, Kim BW, Euler KL, Williams L. Antibacterial and antifungal activities of nine medicinal plants from Zaire. *Int J Pharmacogn* 1994;32:337-45.
4. Kamrani YY, Amanlou M, Esmaeelian B, Rahimi M. *In vitro* antibacterial and antiadherence properties of flavonoid-rich extract of *Pistaciaatlantica* hull against microorganisms involved in dental plaque. *Planta Med* 2007;73:178.
5. Yu HH, Lee DH, Seo SJ, You YO. Anticariogenic properties of the extract of *Cyperusrotundus*. *Am J Chin Med*. 2007;35:497-505.
6. Kang MS, Kang IC, Kim SM, Lee HC, Oh JS. Effect of *Leuconostoc* spp. on the formation of *Streptococcus mutans* biofilm. *J Microbiol* 2007;45:291-6.
7. Zanela NL, Bijella MF, Rosa OP. The influence of mouthrinses with antimicrobial solutions on the inhibition of dental plaque and on the levels of *mutansstreptococci* in children. *Braz Oral Res* 2002;16:101-6.
8. Pires JR, Rossa Junior C, Pizzolitto AC. *In vitro* antimicrobial efficiency of a mouthwash containing triclosan/gantrez and sodium bicarbonate. *Braz Oral Res* 2007;21:342-7.
9. Zanatta FB, Antoniazzi RP, Rösing CK. The effect of 0.12% chlorhexidinegluconate rinsing on previously plaque-free and plaque-covered surfaces: A randomized, controlled clinical trial. *J Periodontol* 2007;78:2127-34.
10. Monfrin RC, Ribeiro MC. *In vitro* evaluation of the effects of antiseptic mouthrinses on the microflora from saliva. *Rev Assoc Paul Cirur Dent* 2000;54:400-7.
11. Siegrist BE, Gusberti FA, Brex MC, Weber HP, Lang NP. Efficacy of supervised rinsing with chlorhexidinedigluconate in comparison to phenolic and plant alkaloid compounds. *J Periodont Res* 1986;21:60-73.
12. Betoni JE, Mantovani RP, Barbosa LN, Di Stasi LC, Fernandes Junior A. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. *MemInstOswaldo Cruz* 2006;101:387-90.
13. Nascimento GG, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz J Microbiol* 2000;31:247-56.
14. Carbajal D, Casaco A, Arruzazabala L, Gonzalez R, Tolon Z. Pharmacological study of *Cymbopogoncitrat* leaves. *J Ethnopharmacol* 1989;25:103-7.
15. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. CLSI document M100-S17. [ISBN 1-56238-625-5]. Pennsylvania, USA: Clinical and Laboratory Standards Institute; 2007.
16. Charles CH, Mostler KM, Bartels LL, Mankodi SM. Comparative antiplaque and antigingivitis effectiveness of a chlorhexidine and an essential oil mouthrinse: 6-month clinical trial. *J Clin Periodontol* 2004;31:878-84.
17. Slot DE, Lindeboom R, Rosema NA, Timmerman MF, van der Weijden GA. The effect of 0.12% chlorhexidine dentifrice gel on plaque accumulation: A 3-day non-brushing model. *Int J Dent Hyg* 2007;5:45-52.
18. Fine DH, Furgang D, Barnett ML, Drew C, Steinberg L, Charles CH, *et al.* Effect of an essential oil-containing antiseptic mouthrinse on plaque and salivary *Streptococcus mutans* levels.

- J Clin Periodontol 2000;27:157-61.
19. Duailibe SA, Gonçalves AG, Ahid FJ. Effect of a propolis extract on *Streptococcus mutans* counts *in vivo*. J Appl Oral Sci 2007;15:420-3.
20. Silva NB, Claudino LV, Neves AS, Costa AC, Valença AM. Evaluation of phytotherapy antimicrobial activity against *porphyromonasgingivalis* and *prevotellamelaninogenica*. Pesq Bras Odontoped Clin Integr 2006;6:167-71.
21. Holetz FB, Pessini GL, Sanches NR. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem Inst Oswaldo Cruz Cruz 2002;97:1027-31.
22. Gebara EC, Lima LA, Mayer MP. Propolis antimicrobial activity against periodontopathic bacteria. Braz J Microbiol 2002;33:365-9.

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